aa1 is S, G, A, D, K, R or T;

aa2 is L, F, I, M or W;

aa3 is F or L;

12 cont.

aa4 is any amino acid;

aa5 is any amino acid; and

aa6 is G or A.

13

7. (twice-amanded) The mathod of claim 1 wherein the antibody composition is obtained by immunizing another individual with a carrier-conjugated peptide comprising the conserved motif of amino acids [sequence].

REMARKS

Claims 1 -12 are pending.

Claims 13 - 39 are withdrawn from consideration.

Applicants respectfully request reconsideration and allowance of the claims, as amended, in view the remarks herein and the other amendments to the application. Before addressing each of the items at pages 2 - 10 of the Office Action, Applicants apprise the Examiner that the Specification has been reviewed for typographical and grammatical errors and appropriate correction made by amendment thereto. Applicants respectfully submit that tha corractions ara only to matters of form and that no new matter is introduced by way of these amendments.

Comments on the Amendments to the Claims

The claims have been amended, as set forth above, to address the issues under 35 USC § 103 and § 112. More particularly, the amendments to the claims ara intended to more

pracisely recite the class of 6-mer end 7-mer polypeptides used in the method of the instant invention. Also, tha amandments ara intended to evan more clearly indicate that the method of passive immunization uses a substantially isolated antibody that recognizes end binds a specific class of polypeptides having a conserved motif of amino acids. This is to better distinguish over antisera obtained from tha immunization of an animal with intact E2 antigen, wherein the antisera purportedly contains antibodies against any and all immunogenic epitopes (Office Action Items 19 & 20 below). Support for the claim amandments is found in tha specification, for example, at page 7, lines 24 - 29, page 24, lines 20 -23, page 25, lines 4 - 7, and Example 2 beginning at page 30. It is believed that no new matter has been added by way of these amendments.

Office Action Item 16: Use of Trademarks

The Examiner has maintained his position with regard to amendment of the specification to provide the generic tarminology for each of the trademarks that appears in the disclosure.

In compliance with the Examiner's request, tha specification has been amended to correct all trademark usage as well as to include the generic terminology for aach of the marks. Applicants submit that no new matter has been introduced in making these amendments.

Office Action Items 17 & 18: Objection to the Specification and Rejection of Claims 1 - 12 Under 35 USC §112, First Paragraph

Tha objection to the specification and tha rejection of claims 1 - 12 under 35 USC §112, first paragraph, stand for the reasons set forth in paragraph 20, sections A - E, of tha Office Action mailed 1/26/95. Applicants' arguments were considered, however they were not deemed persuasive. In summary form, the Office Action maintains that:

Section A

First, the specification does not disclose how to use the instant invention for the treatment of HCV infection in humans by not having shown that the chimp model used in the specification predicts efficacy of the antibodies raised against the conserved motif of amino acids in treating humans. (The selected disclosure of Weiner et al. abstract (1992) is again relied on for this objection and rejection.) Also, the activity of the serum protein TBG as exemplified in Applicants' disclosure would competitively bind to an epitope encompassing the SLF--G motif of HCV, and therefore anti-HCV antibodies administered *in vivo* would be prevented from binding to the conserved motif. Further, the chimp model uses one animal and no negative controls, and, thus, it is unclear as to how to draw a conclusion about the efficacy of the experiment.

Second, there is no evidence of record that HCV infection has ever been treated in humans with passive immunization, and none of the claims read on a method of treating HBV*(sic HCV)*. Furthermore, other viruses (such as HIV) have been refractory to antibody treatment (see Fahey et al., entire document.) Applicants have provided no evidence that the inventive method can be used to treat baboons that are infected with HCV prior to immunizing with the specifically raised antibody. "This is the only use for passive immunization that is known in the art (e.g. use after infection.)"

Section B

Third, Applicants have provided no evidence that any particular antibody preparation against any of the particular amino acid sequences recited in claims 1 - 5 could be used in the method of the invention.

Fourth, Applicants have provided no evidence that any particular antibody against any of the peptides embraced by the claims can be used to treat HCV infection in an individual infected with any isolate of HCV. The disclosure of Weiner et al. (1992) is relied on to support this basis.

Fifth, Applicants have provided no evidence that all of the at least 56,000 peptides encompassed by the formula recited in claims 1 and 2 are immunogenic and can result in the production of antibodies which bind any strain of HCV.

Section C

Sixth, there is no evidence that the antibody that binds the 30-mer peptide in the chimp in Example 3 is derived from the peptides recited in claims 1 - 5 and no evidence as to the binding specificity of this particular antibody with regard to epitopes having 3 to 5 amino acids. Applicants' arguments presented 8/1/95, discussing the results of Example 2 (immune response of goats immunized with 30-mer

peptide) were not deemed persuasive. Furthar, th disclosure do s not stablish a nexus between the immune response of baboons and the immune response of goats.

Saventh, thera is (no) disclosure In the specfication as to which antibodies in tha polyclonal antisera against the 30-mer are responsible for the result seen in Example 3.

Eighth, there Is no evidence that antibodies against the 30-mer do not recognize a conformational epitope not contained in the peptides recited in claims 1 and 2.

Section D

Ninth, Applicants have provided no evidence that the claimed peptides other than tha 30-mer peptida of Example 3 can be used to produce the antibody used in the instant invention and achieve the result seen In Example 3.

Tenth, Applicants have not identified the immunoprotective epitope recognized by the antibodies raised against the Intact 30-mer peptide, nor whether this epitope Is linear or conformational. Hence, it is unclear as to what region of E2HV can be used as Immunogen to produce antibodies used in the invention other than the intact 30-mer.

Section E

Eleventh, Applicants have provided no evidence that the antibody used in Example 3 can be used to prevent HCV when the recipient of the antibody is exposed to any strain other than the strain used in that example. The disclosure of Weiner et al. is relied on to support this basis. Further, thera is no evidence provided in the specification that any one particular antibody against a peptide recited in the claims binds any other peptide encompassed by the formula.

Applicants again respectfully traverse tha numerous purported deficiencies of the disclosure and request reconsideration and withdrawal of the objection and rejection In view of the following remarks. The first paragraph of 35 USC § 112 requires that

> The specification shall contain a written description of the Invention, and of the manner and process of making and using it, In such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use tha sama, and shall set forth the best mode contemplated by the inventor of carrying out his Invention.

Applicants respectfully submit that tha description, as a whole, is sufficiently datailed to enabla one of ordinary skill in the art to practice the presently claimed method of passively immunizing an individual against HCV. Applicants make this assertion in the context of what is required by the relavant case law. To satisfy the law, the disclosure must be clearly and sufficiently definite to guide those skilled in the art to its successful application. *Mineral Separation Ltd. v. Hyde*, 242 U.S. 261, 271 (1916). *In re Marzocchi*, 169 USPQ 367 (CCPA 1971), holds that first paragraph of § 112 requires nothing mora than objective anablement and how this is accomplished is of no importance.

Again, Applicants direct the Examiner's attention to the evidence in the disclosure, as originally filed, to indicata that ona of skill in tha art would *not* doubt the scopa of anablement. More specifically, the specification provides a description of

(1) the identification and production of polypeptides having a conserved motif of amino acids from the E2/NS1 antigan of hepatitis C virus (a.g., the disclosure at pages 15 - 18 and Ex. 1 and Figs. 2 - 4);

(2) tha production of mono- and poly- clonal antibodies raised against the polypeptida characterized by a conserved motif of amino acids, as well as the preparation of an antibody-IgG composition (a.g., the disclosure at pages 23 - 25 and Ex. 2, beginning at paga 30);

(3) a method of using antibodies raised against the polypeptides having a conserved motif of amino acids in passive immunization against HCV (e.g., the disclosure at pages 25 and Ex. 3); and

(4) a method of using these antibodies to detect HCV antigen in a biological sampla (a.g., the disclosure at pages 25 - 26).

Applicants have satisfied what is required by law to establish sufficient disclosure, namely, setting forth how to make and use the invention, setting forth the best mode, and demonstrating that the invantion works. Moreover, sufficient reasons for doubting tha truth or accuracy of

assertions made in the specification must be provided before Applicants are obligated to show evidence supporting the truth or accuracy of the claimed invention.

As to *Section A, First* and *Second,* tha lack of human data is insufficient to establish a rejection undar 35 USC § 112, first paragraph. Section 112 does not requlre that axperimentation actually be performed on human subjects. Even the PTO Legal Analysis in support of the Utility Examination Guidelines (60 FR 36263, July 14, 1995) reiterates this point, stating

> "Office personnel should not lmpose on applicants tha unnecessary burden of providing evidence from human clinical trials. There is no decisional law that requires an applicant to provide data from human clinical trials to establish utility for an lnvantion related to the treatment of human disorders aven with respect to situations where no art-recognized animal models exist for the human disease encompassed by the claims (Section III, D. Human Clinlcal Trials ,citing *Ex parte Balzarini*, 21 USPQ2d 1892 (Bd. Pat. App. & Int. 1991)).

The PTO Legal Analysis states further that tha courts hava repeatedly held that a reasonable correlation, and not statistical certainty, between tha activlty In question and the asserted use is all that Is requlred (Section III, A. A Reasonable Correlatlon Between Evidence and the Asserted Utility is Sufficient, citing *Nelson v. Bowler*, 206 USPQ 881, 884 (CCPA 1980)). If one skilled in the art would accept the anlmal tests as being reasonably predictive of utility In humans , evidence from those tests should be considered sufficient to support the credibility of the asserted utility. (Section III, C. Data From In Vitro or Animal Testing is Generally Sufficient to Support Therapeutlc Utility, citing *In re Krimmel*, 130 USPQ 215, 219 (CCPA 1961)(defining "standard experimental animals" as "whatever animal is usually used by those skilled in the art to establish the particular pharmaceutical application in question")).

A more recent, relevant decislon that carries a higher degrea of persuasiva authority than tha decisions cited hereinabove is tha Federal Circuit's ruling in *In re Brana*, 34 USPQ2d

1436 (Fed. Cir. 1995). There, the Federal Circuit overruled the Patent Office for failing to avaluate evidance provided by tha applicant with tha proper lavel of def renca and rejected the PTO's requirement of a higher standard of proof of therapeutic utility. *In re Brana* states in part

> "... The Commissioner, as did the Board, confuses tha requirements under the law for obtaining a patent with the requirements for obtaining government approval to market a particular drug for consumption. See *Scott* v. *Finney*, ..., 32 USPQ2d 1115, 1120 (Fed. Cir. 1994) ("Testing for the full safety and affectivanass of a prosthetic device is more properly laft to the Food and Drug Administration (FDA). Title 35 does not demand that such human testing occur within the confines of Patent and Trademark Office proceedings.")" *In re Brana*, at 1442.

Applicants incorporate herein the remarks conceming the dearth of suitable animal models for testing HCV therapeutics and prophylactics, as presented at page 10 of their previous response. To stress what is perhaps the most important point of those earlier remarks, i.e., that the chimpanzee is the only reliable model recognized in the field, a Declaration Under 37 CFR 1.132 is attached averring to this fact. Exhibit B of the Declaration (Choo et al.) provides further avidence that the chimp model is accepted in the field of HCV research. The Declaration also avers that the passive immunization test results of tha chimpanzee of Example 3 reasonably correlate to, or are reasonably predictive of, the asserted utility in humans. Turning again to the PTO Legal Analysis Supporting the Utility Examination Guidelines,

> "Evidence doas not have to be in the form of data from an art-recognized animal model for the particular disease or disease condition to which the asserted utility relates. Data from any test that the applicant reasonably correlates to the asserted utility should be evaluated substantively."

(Section III, C. Data From In Vitro or Animal Testing is Generally Sufficient to Support Therapeutic Utility). It is respectfully submitted that Applicants' evidence of record of the therapeutic utility, as demonstrated in the chimpanzee model and the other examples, and further in conjunction with the attached Declaration satisfies both utility and enablement requirements.

As to serum TBG, th re is no vidence in the record suggesting that in the presently disclosed method of passively immunizing a human against HCV, the conserved motif of amino acids of E2HV region of the E2/NS1 antigen of HCV would preferably bind to serum TBG over anti-HCV antibodies. Nor is there any evidence of record to suggest that serum TBG would competitively prevent binding of anti-HCV antibodies to the conserved region. Thus, this rejection, if proper at all, appears to be based upon facts within the personal knowledge of the Examiner. If this rejection is maintained, the Examiner is requested to provide evidence to support this ground of rejection pursuant to 37 CFR § 1.107(b).

Similarly, the requirement of providing controls in order to assess the therapeutic results observed in Example 3 is beyond the scope of what the law requires and the guidelines recommend. Neither statistical certainty nor actual evidence of success in treating humans is required. Rigorous correlation is not necessary when the test is reasonably predictive of the response. *Nelson v. Bowler*, 206 USPQ 881, 883 - 884 (CCPA 1980). The protective effect of the passively administered antibody was measured qualitatively in Example 3 (e.g., by the absence of abnormalities in liver function and histology and other overt symptoms) following infection with not one, but 10 chimpanzee infective doses of hepatitis C virus. No signs of viral replication were detected where viral replication would otherwise have been inevitable given the infective dose. It is respectfully that this evidence is reasonably predictive of the asserted utility.

Furthermore, that "there is no evidence of record that HCV infection in humans has ever been treated with passive immunization" (Office Action, page 4) is doubt beyond the scope of § 112, first paragraph. In fact, Applicants are not required to provide evidence sufficient to establish that an asserted utility is true beyond a reasonable doubt. *In re Irons*, 144 USPQ 351, 354 (1965). Rather, the evidence is sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely than not true. (PTO Legal Analysis Supporting the Utility Examination Guidelines, Section II, F. Evaluation of

Evidence Related to Utility) There is no reason to beliave, and none exists in the record, that tha protectiva affact observed in Example 3 will not also occur in human subj cts. Thus, it appears that tha rejection is based upon facts within the personal knowledge of the Examiner. The Examiner is requested to provide evidence to support this ground of rejection pursuant to 37 CFR § 1.107(b).

Nota that very recently, enablement was found to be sufficient in a situation whare one taught the public that a compound exhibits soma desirabla pharmaceutical property in a standard experimental animal and thus had mada a significant and useful contribution to the art, aven though it may aventually appear that tha compound is without valua in the treatment of humans. *In re Brana*, 34 USPQ2d 1436, 1442 (CAFC 1995)(citing *In re Krimmel*, at 219). Thus, hypothetically speaking, even if upon testing anti-HCV antibodies in human clinical trials tha protective affect of passive immunization, observed in the chimp model, is not as promising in human subjects, *In re Brana* indicates that this will not negate the sufficiency of the enablement. The result seen in Applicants' Example 3, as well as in the other examples, constitutes a significant and useful contribution to the art.

Applicants disagree with the reasoning in the Office Action that attempts to place HCV in the "same" category with HIV. In particular, reference was made to Fahey et al. for disclosing that HIV is refractory to antibody treatment, and, hence, this is sufficient basis for doubting Applicants' utility of passiva immunization with anti-HCV antibodies. HIV and HCV are dissimilar viruses and are not taxonomically related as they are in two different virus families, Retroviridae and Flaviviridae, respectively. Although both HCV and HIV are RNA viruses, the mode of viral replication for aach is different. Retroviruses such as HIV replicate from RNA to DNA to mRNA. HCV does not translate to DNA during replication evidenced by the absence of reverse transcriptase. Rather the single strand RNA genome of HCV is transcribed directly into protein. HIV is further characterized by having many different reading frames, whereas HCV is a long

polyprotein with a single reading frame. Accordingly, the two viruses are disparate in their replication patterns and protein composition, such that the results of HIV research are not predictive of the anticipated success or failure of antibody-based therapy for HCV. Hence, it is respectfully submitted that reliance on Fahey et al. to support an enablement rejection is not proper.

Applicants are not required under 35 USC § 112, first paragraph, to provide evidence of a previously HCV -infected chimpanzee being treated with anti-HCV antibodies, as suggested by the Examiner, as proof of the passive immunization presently claimed. Moreover, Applicants disagree with the statement "This is the only use for passive immunization that is known in the art (e.g. use after infection.)" It is well-established in the field of immunology that passive immunization can be used prophylactically, that is, prior to or immediately after exposure. (See attached Barrett, J., Basic Immunology and its medical application , 2nd Ed., pg. 17 (1980).) Passive immunization can also be used less efficiently in therapeutic applications. Ibid. Thus, the protocol used in Example 3, in which the uninfected chimpanzee was first administered anti-E2HV sheep IgG preparation followed by administration of 10 chimpanzee-infective doses of HCV, is properly a passive immunization protocol for a prophylactic therapy. Moreover, the law does not require that each and every embodiment of an invention be exemplified to satisfy the enablement requirement. An applicant need not provide a specific example of everything embraced by a broad claim. *In re Anderson* (CCPA 1973) 176 USPQ 331. In fact, Applicants have exemplified various aspects of the invention, namely, the identification and production of polypeptides having a conserved motif of amino acids from the E2/NS1 antigen of hepatitis C virus (e.g., the disclosure at pages 15 - 18 and Ex. 1 and Figs. 2 - 4), the production of mono- and poly- clonal antibodies raised against the polypeptide characterized by a conserved motif of amino acids, as well as the preparation of an antibody-IgG composition (e.g., the disclosure at pages 23 - 25 and Ex. 2, beginning at page 30), a method of using antibodies raised against the polypeptides having a conserved motif of amino acids in passive immunization against HCV

(e.g., the disclosure at pages 25 and Ex. 3), and a method of using these antibodies to detect HCV antigen in a biological sample (e.g., the disclosure at pages 25 - 26).

The individual bases of *Section B, Third, Fourth* and *Fifth* and *Section E, Eleventh* are again traversed. First, all of the preceding remarks and case citations which address the issue of enablement requirements apply equally to these bases. Second, there is no requirement that each embodiment of an invention be operable and exemplified. The inclusion of nonoperable embodiments is not a bar to patentability. There is no requirement in Section 112 that all of the claimed compounds (encompassed by a generic claim) must possess the same degree of utility. *In re Gardner* (CCPA 1973) 224 USPQ 409. The mere fact that routine experimentation might be required to determine whether any particular embodiment would be useful in the claimed method alone is not sufficient reason to deny claims to the method which utilizes antibodies raised against a class of 140 conserved peptides. See *Atlas Power Co. v. E.I. Du Pont De Nemours & Co.* (CAFC 1984) 224 USPQ 409. Also, it is not the function of the claims to specifically exclude possible inoperative substances. *Id.*

Therefore, Applicants maintain that they have no legal obligation to show that antibodies to any of the claimed peptides would bind to any HCV isolate. It is well within the routine skill of one in the art to make that determination, especially in view of the fact that antibodies used in the method of the invention recognize and bind to a specific conserved motif of amino acids in a large number of HCV isolates. This fact leads one of ordinary skill in the art to the reasonable expectation that the antibodies will bind this region in other isolates.

Moreover, contrary to the Examiner's assertions regarding Weiner et al., Weiner et al. do not teach against the present invention. There is no data or teaching provided in the article that indicat that th antibodies in the on patient described were directed against the conserved

motif of amino acids as required by tha present Invention. Also, the presently claimed Invention is to a method of passive immunization, that is, administering anti-HCV antibodies before axposure to HCV or immediately thereafter (before the virus replicates.) Passive immunization is classified as prophylactic, for reasons stated earlier. The study in Weiner et al. was of a chronically-infected individual, and thus, the treatment would be a therapeutic one, not prophylactic. Administering antiserum alone therapeutically to a chronically-infected individual would not be expected to repair existing cell damage caused by the virus(or its toxins) with an established foothold of replication in the host. (See Barrett, J., Basic immunology and its medical application , 2nd Ed., pg. 17.) Accordingly, the disclosure of Weiner et al. is not seen as providing the factual support required for the Examiner's doubts about the sufficiency of enablement.

The bases for objection and rejection in *Section C* are again traversed. It is noted that the Examiner continues to look to the 56,000 peptides embraced by the Sequence Listing and maintains the requirement that Applicants provide evidence that all 56,000 are immunogenic and yield anti-HCV antibodies that will bind to any HCV isolate.(*Section C, Sixth*) Again, this requirement is believed to be outside tha scope of § 112, first paragraph. Applicants incorporate herein their previous remarks traversing this basis of objection and rejection, at pages 13 - 14 of the Amendment filed 8/1/95. To stress the salient points, Applicants point out that not all positions in the peptide are relevant to the invention. Certain amino acid residues are irrelevant to the conserved motif. (e.g., residues 4 and 5 of the 6-mer peptide). Therefore, the actual number of combinations available is calculable as the product of the possible residues at each of the critical positions (i.e., 1, 2, 3, and 6). Thus the maximum number of combinations of the critical residues allowed in claim 1 is 7 x 5 x 2 x 2 = 140. For the other claimed preferred embodiments the maximum number of combinations of the critical residues are 420 (claim 2), 4 (claim 3), 8 (claim 4), and 1 (claim 5). Furthermore, Section 112 does not require that each and

evary species of tha prefarred embodimants be tested, since to do so would frustrate rather than advance the public interest. *In re Bundy*, 209 USPQ 48, 52 (CCPA 1981).

Applicants disagrea that they are required to show some nexus between the immune response of sheep and that of the baboon. It is weil-established in the fieid of immunoiogy that the source of immunogiobulins for artificiai passiva immunity in humans is either from another human or from a lower animai. (See Barrett, J., Basic immunology and its medicai application , 2nd Ed., pp. 15 - 16, attached). Barrett reports that severai pharmaceutical companies are involved in iarge-scale antibody production in horses, cows, and other animais by active immunization, so that the antibodies may be iater used in humans to prevent human diseases by passive immunization. *Ibid.*,pg. 16. There is no reason to believe, and none exists in the record, that sheep IgG containing anti-HCV antibodies are not an acceptabla source of antibodies for passive immunization of a higher primate, such as a chimpanzee or a human being. Nor is there any reason to believe that one having ordinary skili, having the present disclosure in hand, wouid not reasonably predict that the observed binding specificity of these sheep antibodies to the region of the conserved peptide (Example 2) wouid also occur in the chimp or a human. Absolute predictabiiity is not required by the patent iaws. Thus, it appears that the rejection is based upon facts within tha personal knowiedge of the Examiner. The Examiner is requested to provide evidence to support this ground of rejection pursuant to 37 CFR § 1.107(b).

Section C, Seventh and *Section D, Ninth* deai primarily with the 30-mer peptide of Example 3, the antibodies raised against it, antibody binding specificity, and the protective immunization effect achieved therewith. Applicants consider the remarks in the preceding paragraph which relate to Exampie 2 to be relevant herein. Example 2 establishes through several overiapping peptides of the 30-mer synthesized that the antibody binding observed was consistently to a conserved region iocated between amino acid residues 401 - 407. It is precisely to this conserved peptide region and the antibody raised against it that th claimed

subject matter is directed. Henca, the Examiner's statements that 1) there is no disclosure in the specification as to which antibodies in tha polyclonal antisera against tha 30-mar are responsible for tha result sean in Example 3, and 2) no evidence has been provided showing that tha claimed peptides, othar than the 30-mer peptida of Exampia 3, can be used to produce the inventive antibody and achieve the result seen in Example 3, appears to be in total disregard of the enabling disclosure of Examples 1, 2, and 3, and the remainder of the application disclosura. It is respectfully submitted that tha disclosure as a whole gives rise to a reasonable axpectation that the limited genera of conserved peptides recited in the claims, and the antibodies raised therefrom, would function as alleged and as demonstrated. There is no factual avidence of record to tha contrary.

As to the purported failure of the disclosure to identify whether tha immunoprotactiva epitope recognized in Example 3 is linear or conformational (*Section C, Eighth* and *Section D, Tanth*) Applicants again traverse this basis for objection and rejection. Again, it is respectfully submitted that this basis is outside the scope required by § 112, first paragraph. Applicants' disclosure identifies a polypeptide characterized by a specified conserved amino acid pattern, and shown to be immunogenic. This discovery arose from Applicants elucidating that from isolates of 90 different HCV strains, the E2/NS1 antigenic region contains not only a hypervariable region between variants, but surprisingly, a conserved peptide region as well. (Example 1 of the specification) Moreover, the disclosure sufficiently teaches one of skill in the art how to use these polypeptides in the raising of antibodies which are also useful in various modes of treating and diagnosing HCV in humans. More particularly, the disclosure exemplifies how these antibodies are used in a prophylactic treatment, i.e., passive immunization, and demonstrate an effective qualitative result. It is to this utility that the present claims are directed. Applicants have therefore taught how to make and use the invention within the strictures of § 112, first paragraph, and believe that one of ordinary skill in the art would not doubt the scope of enablement. Once again, it is respectfully submitted that sufficient reasons

for doubting the truth or accuracy of assertions made in the specification must be provided before Applicants are obligated to show evidence supporting the truth or accuracy of the claimed invention.

Reconsideration and withdrawal of the objection and rejection under 35 USC § 112, first paragraph, is respectfully requested.

Office Action Items 19 & 20: Rejection of Claims 1 - 12 Under 35 USC § 103 Over Ralston et al. in view of Houghton et al.

Claims 1 - 12 stand rejected under 35 USC § 103 Over Ralston et al. in view of Houghton et al. The Office Action states, in part

> "Ralston et al. teach that regarding the E2 antigen 'immunogenic compositions may be administered to animals to induce production of antibodies, either to provide a source of antibodies or to induce protective immunity in the animal' (page 15, lines 15 - 19). The animal immunized with intact antigen would produce a polyclonal antisera containing antibodies against any and all immunogenic epitopes expressed on said molecule (e.g. including the conserved motif recited in claim 1)."

Applicants respectfully traverse the rejection. Neither Ralston et al. nor Houghton et al. disclose or suggest the immunogenic 6-mer and 7-mer peptide having the conserved motif of amino acids as discovered by Applicants. Moreover, absent hindsight, there is no motivation in the individual or combined teachings of the cited art to single out the recited conserved 6-mer and 7-mer from the tremendously large number of unconserved 6- (64,000,000) and 7-mers(1,280,000,000) in the intact E2 antigen. Further, absent some clear direction in the cited art, there is no motivation to single out the particular antibodies raised against these conserved peptides from an antiserum produced from immunization with the intact E2 antigen and containing antibodies against any and all epitopes. Clearly to select Applicants' conserved peptide, and the antibodies raised thereagainst, from the generic teachings at pag 15 of Ralston

et al. would require excessive experimentation. It is submitted that, at best, it would be "obvious to try" and this is not the standard for a valid § 103 rejection.

Additionally, tha rejection maintains that because tha claim 1 recites a mathod of using an antibody compositlon "comprising," the purported mixture of antibodies disclosed in Ralston et al. constitutas prior art. Claim 1 has been amended to recite a mathod of passive immunization using a substantially isolated antibody that recognizes and binds the class of polypeptides having the specified conserved motif of amino acids. Applicants intend by the term "substantially isolated antibody" an antibody raised against the conserved motif of amino acids, as exemplified in Example 2 of the application. Neither the claims nor the disclosure as originally filed were ever directed to mixtures of antibodies from antisera resulting from immunization with tha intact E2 antigen. The disclosure at, for example, page 7, lines 24 - 29, page 24, lines 20 -23, page 25, linas 4 - 7, and Exampla 2 beginning at page 30, is evidence of this and is believed to be support for the claim amendment. Accordingly, it is respectfully submitted that the claimed subject matter herein is even further distinguished over Ralston et al.. Reconsideration and withdrawal of the rejection are respectfully requested.

Office Action Items 21, 22 & 23: Objection to the Specification and Rejection of Claims 1 - 12 Under 35 USC § 112, First Paragraph

The specification was newly objected to and tha claims newly rejected under 35 USC § 112, first paragraph, on the basis that the term "conserved amino acid sequence" in claims 1 and 7 (both amended) was not supported in the specification as originally filed. The Office Action noted the disclosure at page 7, last paragraph, as providing support for the terminology " a conserved motif of amino acids," meaning a conserved pattern of amino acids, and not a particular conserved amino acid sequence.

In an effort to more precisely claim the invention, Applicants have amended claims 1 and 7 herein to recite that a substantially isolated antibody recognizes and binds to a conserved motif of amino acids having the specified formula. Withdrawal of the objection and rejection are believed to be proper and are respectfully requested.

Favorable reconsideration and allowance of all the pending claims, in view of the above amendments, remarks and the the attached Declaration Under 37 CFR 1.132, are earnestly sought.

In the unlikely event that the transmittal letter is separated from this document and the Patent Offica determines that an extension and/or other relief is required, Applicants' petition for any required, including extensions of time, and authorize the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to our Depoist Account No. 03-1664. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

Date: 5-6-96

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EXHIBIT A

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PUBLICATIONS

1. Fausto-Sterling A, Weiner AJ, Digan ME. Analysis of a newly-isolated temperature-sensitive maternal-effect mutation of Drosophila melanogaster. J. Exper. Zoo. 1976; **200**: 199-210.

2. Guntaka RV, Weiner AJ. Effect of dibutyrlcyclic AMP on intracellular levels of avian sarcoma virus specific RNA. Nature 1978; **274**: 274-276.

3. Guntaka RV, Katz RA, Weiner AJ, Widman MJ. Effect of 5-methylcytidine on virus production in avian sarcoma virus-infected chicken embryo cells. Virology 1979; **29**: 475-482.

4. Brennan M, Weiner AJ, Goralski TJ, Mahowald AP. The follclle cells are a major site of vitellogenin in Drosophila melanogaster. Dev. Biol. 1982; **89**: 225-236.

5. Scott MP, Weiner AJ, Hazelrigg T, Schelenghe F, Pirrota V, Kaufmann TC. The molecular organization of the Antennapedia Complex in Drosophila melanogaster. Cell 1983; **35**: 763-776.

6. Scott MP, Weiner AJ. Structural relationships among genes that control development: sequence homology between the Antennapedia, Ultrabithorax and fushi tarazu loci of Drosophila melanogaster. PNAS 1984; **81**: 4115-4119.

7. Weiner AJ, Scott, MP, Kaufman, TC. A molecular analysis of the fushi tarazu locus, a gene whose product is required for segment number and cell fate in Drosophila melanogaster. Cell 1984; **37**: 843-851.

8. Wang K-S, Choo Q-L, Weiner AJ, Ou J-H, Najarian RC, Thayer RM, Mullenbach GT, Denniston KJ, Gerin JL, Houghton M. Structure, sequence and expression of the hepatitis delta (d) viral genome. Nature 1986; **323**: 508-514.

9. Houghton M, Weiner AJ, Wang KS, Choo Q-L. Hepatitis delta virus HDV: Its relationship with introns and plant viroid-like agents and the mapping of immunogenic epitopes within viral polypeptides. J. Med. Virol 1987; **21**: 37.

10. Wang K-S, Choo Q-L, Weiner AJ, Ou J-H, Denniston KJ, Gerin JL, Houghton M. The viroid-like structure of the Hepatitis Delta (δ) genome: synthesis of a viral antigen in recombinant bacteria. In: Rizzetto M, Gerin JL, Purcell RH, eds. Progress in Clinical and Biological Research: The Hepatitis Delta Virus and its Infection, 24, New York: Alan R. Liss, 1987: 71-82.

11. Weiner AJ, Wang K-S, Choo Q-L, Gerin JL, Bradley DW, Houghton M. Hepatitis delta (d) cDNA clones: undetectable hybridization to nucleic acids from infectious Non-A, Non-B Hepatitis materials and Hepatitis B DNA. J. Med. Virol. 1987; **21**: 239-247.

12. Weiner AJ, Choo Q-L, Wang K-S, Govindarajan S, Redeker AG, Gerin JL, Houghton M. A single antigenomic open reading frame of the hepatitis delta virus encodes the epitope(s) of both hepatitis delta antigen polypeptides p24 and p27. J. Virol. 1988; **62**: 594-599.

13. Houghton M, Richman K, Han J, Berger K, Lee C, Dong C, Overby L, Weiner A, Bradley D, Kuo G, Choo Q-L. In Viral Hepatitis and Liver Disease. (Ed. AJ. Zuckerman. New York: Alan R. Liss) Hepatitis C virus (HCV): A relative of the pestiviruses and flaviviruses. 1988.

14. Choo Q-L, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989; **244**: 359-362.

15. Kubo Y, Takeuchi K, Boonmar S, Katayama T, Choo Q-L, Kuo G, Weiner AJ, Bradley DW, Houghton M, Saito I, Miyamura T. A cDNA fragment of hepatitis C virus isolated from an implicated donor of post-transfusion non-A, non-B hepatitis in Japan. Nucl. Acids Res. 1989; **17**: 10367-10372.

16. Choo Q-L, Weiner AJ, Overby LR, Kuo G, Houghton M, Bradley DW. Hepatitis C virus: The major causative agent of viral non-A, non-B hepatitis. Br. Med. Bull. 1990; **46**: 423-441.

17. Shimizu YK, Weiner AJ, Rosenblatt J, Wong DC, Shapiro M, Popkin T, Houghton M, Alter HJ, Purcell RH. Early events in hepatitis C virus infection of chimpanzees. Proc. Natl. Acad. Sci. USA 1990; **87**: 6441-6444.

18. Weiner AJ, Kuo G, Bradley DW, Bonino F, Saracco G, Lee C, Rosenblatt J, Choo Q-L, Houghton M. Direct detection of hepatitis C viral sequences in non-A, non-B hepatitis. Lancet 1990; **335**: 1-3.

19.Weiner AJ, Truett MA, Rosenblatt J, Han J, Quan S, Polito AJ, Kuo G, Choo Q-L, Houghton M, Aglus C, Page E, Nelles MJ. HCV testing in a low-risk population. Lancet 1990; **336**: 695.

20.Choo Q-L, Han J, Weiner AJ, Overby LR, Bradley DW, Kuo G, Houghton M. Hepatitis C virus is a distant relative of the flavivirus and pestivirus. In: Shikata T, Purcell RH, Uchida T, eds. Proceedings of 1989 International Symposium on Viral Hepatitis C, D and E, Nihon University, Japan. Amsterdam: Elsevier, 1990.

21.van der Poel CL, Cuypers HTM, Reesink HW, Weiner AJ, Polito A, Van Boven JJP, Winkel I, Mulder-Folkerts D, Exel-Oehlers PJ, Schaasberg W, Leentvaar-Kuypers A, Houghton M, Lelie PN. Confirmation of hepatitis C virus infection by new four-antigen recombinant immunoblot assay and polymerase chain reaction. Lancet 1990; **337**: 317-319.

22.Weiner AJ, Truett MA, Rosenblatt J, Han J, Quan S, Polito AJ, Kuo G, Choo Q-L, Houghton M. HCV: Immunologic and hybridization-based diagnostics. In: Viral Hepatitis and Liver Disease (Hollinger BF, Lemon SH, Margolis H, eds., Williams & Wilkins, 1991).

23.Weiner AJ, Brauer MJ, Rosenblatt J, Richman KH, Tung J, Crawford K, Bonino F, Saracco G, Choo Q-L, Houghton M, Han JH. Variable and hypervariable domains found in the regions of HCV corresponding to the flavivirus envelope and NS1 proteins and the pestivirus envelope glycoproteins. Virol. 1991; **180**: 842-848.

24.Choo Q-L, Richman K, Han JH, Berger K, Lee C, Dong C, Gallegos C, Coit A, Medina-Selby A, Barr PJ, Weiner AJ, Bradley DW, Kuo, G, Houghton M. Genetic organization and diversity of the Hepatitis C virus. PNAS 1991; **88**: 2451-2455.

25.Houghton M, Richman K, Han J, Berger K, Lee C, Dong C, Overby L, Weiner A, Bradley D, Kuo G, Choo Q-L. Hepatitis C virus (HCV): A relative of the pestiviruses and flaviviruses (Baltimore, MD: Williams & Wilkins). 1991.

26.Houghton M, Weiner A, Han J, Kuo G, Choo Q-L. Molecular biology of the hepatitis C viruses: Implications for the diagnosis, development and control of viral disease. Hepatology 1991; **14**: 381-388.

27.Cuypers HTM, Winkel IN., Van der Poel CL, Reesink HW, Lelie PN, Hougton M, Weiner A.. Analysis of genomic variability of hepatitis-C virus. Hepatology 1991; **13**: S15-S19.

28Weiner AJ, Christopherson C, Hall JE, Bonino F, Saracco G, Crawford K, Marion CD, Crawford KA, Venkatakrishna S, Miyamura T, McHutchinson J, Cuypers T, Houghton M. Sequence variation in hepatitis C viral isolates. Hepatology 1991; **13**: S6-S14.

29.Weiner AJ, Geysen HM, Christopherson C, Hall JE, Mason TJ, Saracco G, Bonino F, Crawford K, Marion CD, Crawford KA, Brunetto M, Barr PJ, Miyamura T, McHutchinson J, Houghton M. Evidence for immune selection of hepatitis C virus (HCV) putative envelope glycoprotein variants: Potential role in chronic HCV infections. Proc. Natl. Acad. Sci. USA 1992; **89**: 3468-3472.

30.Weiner AJ, Geysen HM, Christopherson C, Hall JE, Mason TJ, Saracco G, Bonino F, Brunetto M, Crawford K, Marion CD, Crawford KA, Barr PJ, Miyamura T, McHutchinson J, Richman K, Kuo G, Houghton M. The hypervariable amino terminus of the hepatitis C virus (HCV) E2/NS1 protein appears to be under immune selection. CSH Vaccines '92, pp 303-308 (Cold Spring Harbor Press, New York; Ed. F. Brown, R. Chanock, H. Ginsberg, R. Lerner).

31.Martell M, Esteban JI, Quer J, Genescá J, Weiner A, Esteban R, Guardia J, Gómez J. HCV circulates as a population of different but closely related genomes (quasispecies nature of HCV-genome distribution). J. Virol. 1992; **66**: 3225-3229.

32.Choo Q-L, Kuo G, Weiner A, Wang K-S, Overby L, Bradley D, Houghton M. Identification of the major, parenteral non-A, non-B hepatitis agent (Hepatitis C Virus) using a recombinant cDNA approach. Seminars in Liver Disease 1992; **12**:279-288.

33.Cuypers HT, Bresters D, Winkel IN, Reesink HW, Weiner AJ, Houghton, M, van der Poel CL, Lelie PN. Storage conditions of blood samples and primer selection affect the yield of cDNA polymerase chain reaction products of hepatitis C virus. J. Clin. Microbiol. 1992; **30**: 3220-3224.

34.Martell M, Esteban JI, Quer J, Genesca J, Weiner A, Esteban R, Guardia J, Gomez J. Hepatitis C virus (HCV) circulates as a population of different but closely related genomes: Quasispecies nature of HCV genome distribution. J. Virol. 1992; **66**: 3225-3229.

35.Weiner AJ, Venkatakrishna S, Hall JE, Houghton M, Han J. PCR: Application to hepatitis C virus (HCV) research and diagnostics. In Frontiers in Virology, 1992, pp 86-100, (Springer Verlag, New York; Ed. Y. Becker, G. Darai).

36.Botarelli P, Brunetto MR, Weiner AJ, Minutello MA, Unutmaz D, Calvo P, Bonino F, Houghton M, Abrignani S. T cell response to recombinant proteins of hepatitis C virus in blood and liver of patients with different clinical courses of infection. Gastroenterology 1993; **104**: 580-587.

37.Erickson AL, Houghton M, Choo Q-L, Weiner AJ, Ralston R, Muchmore E, Walker CM. Hepatitis C virus-specific CTL responses in the liver of chimpanzees with acute and chronic hepatitis. C. J. Immunol. 1993; **151**: 4189-4199.

38.Weiner AJ, Thaler MM, Crawford, K, Ching K, Kansopon J, Chien DY, Hall JE, Hu F, Houghton M. A unique, predominant hepatitis C virus variant found in an infant born to a mother with multiple variants. J. Virol. 1993; **67**: 4365-4368.

39.Saracco G, Rosina F, Abate ML, Chiandussi L, Gallo V, Cerutti E, De Napoli A, Solinas A, Deplano A, Tocco A, Cossu P, Chien D, Kuo G, Polito A, Weiner AJ, Houghton M, Verme G, Bonino F, Rizzetto M. Long-term follow-up of patients with chronic hepatitis C treated with different doses of interferon-α_{2b}. Hepatology 1993; **18**: 1300-1305.

40.Choo Q-L, Kuo G, Ralston R, Weiner A, Chien D, Van Nest G, Han J, Berger K, Thudium K, Kuo C, Kansopon J, McFarland J, Tabrizi A, Ching K, Moss B, Cummins L.B, Houghton M, Muchmore E. Vaccination of chimpanzees against infection by the hepatitis C virus. Proc. Natl. Acad. Sci. USA 1994; **91**: 1294-1298.

42.Simmonds P, Alberti A, Alter HJ, Bonino F, Bradley DW, Brechot C, Brouwer JT, Chan S-W, Chayama K, Chen D-S, Choo Q-L, Colombo M, Cuypers HTM, Date T, Dusheiko GM, Esteban JI, Fay O, Hadziyannis SJ, Han J, Hatzakis A, Holmes ED, Hotta H, Houghton M, Irvine B, Kohara M, Kolberg JA, Kuo G, Lau JYN, NLelie PN, Maertens G, McOmish F, Miyamura T, Mizokami M, Nomoto A, Prince AM, Reesink HW, Rice C, Roggendorf M, Schalm SW, Shimotohno K, Stuyver L, Trépo C, Weiner A, Yap PL, Urdea MS. A proposed system for the nomenclature of hepatitis C viral genotypes. Hepatology 1994; **19**: 1321-1324.

43.Houghton M, Choo Q-L, Kuo G, Ralston R, Weiner A, Chien D, Seiby M, Han J, Walker C, Abrignani S, Koziel M, Walker B, Cummins L, Muchmore E. The hepatitis C virus: Genetic organization, persistence, and vaccine strategies. In: Nishioka K, Suzuki H, Mishiro S, Oda T, (eds.): Viral Hepatitis and Liver Disease 1994; Springer-Verlag, Tokyo, pp. 33-37.

44.Weiner AJ, Thaler MM, Crawford K, Kansopon J, Ching K, Hall JE, Hu F, Chien D, Houghton M. HCV-positive, HIV-1-negative mothers transmit HCV. In: Nishioka K, Suzuki H, Mishiro S, Oda T, (eds.): Viral Hepatitis and Liver Disease 1994; Springer-Verlag, Tokyo, pp.463-467.

45.Houghton M, Selby M, Weiner A, Choo Q-L. Hepatitis C virus: Structure, protein products and processing of the polyprotein precursor. Curr. Stud. Hematol. Blood Transfus. (Switzerland) 1994; **61**:1-11.

46.Weiner A, Erickson AL, Kansopon J, Crawford K, Muchmore E, Hughes AL, Houghton M, Walker CM. Persistent hepatitis C virus infection in a chimpanzee is associated with emergence of a cytotoxic T lymphocyte escape variant. Proc. Natl. Acad. Sci. USA 1995; **92**: 2755-2759.

47.Weiner, AJ. Humoral response to linear B cell epitopes in the amino terminus of the hepatitis C virus envelope glycoprotein gp72 (E2): Role in protective immunity still unknown. Hepatology 1995; **22**: 369-371.

EXHIBIT B

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Vaccination of chimpanzees against infection by the hepatitis C virus

(immunization)

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ABSTRACT A high incidence of community-acquired hepatitis C virus infection that can lead to the progressive development of chronic active hepatitis, liver cirrhosis, and primary hepatocellular carcinoma occurs throughout the world. A vaccine to control the spread of this agent that represents a major cause of chronic liver disease is therefore needed. Seven chimpanzees (*Pan troglodytes*) have been immunized with both putative envelope glycoproteins [E1 (gp33) and E2 (gp72)] that were copurified from HeLa cells infected with a recombinant vaccinia virus expression vector. Despite the induction of a weak humoral immune response to these viral glycoproteins in experimentally infected chimpanzees, a strong humoral immune response was obtained in all vaccinees. The five highest responders showed complete protection against an i.v. challenge with homologous hepatitis C virus 1. The remaining two vaccinees became infected, but both infection and disease may have been ameliorated in comparison with four similarly challenged control chimpanzees, all of which developed acute hepatitis and chronic infections. These results provide considerable encouragement for the eventual control of hepatitis C virus infection by vaccination.

The hepatitis C virus (HCV) was characterized in 1989 (1) and has been shown (2, 3) to be the major etiological agent of parenterally transmitted, viral non-A, non-B hepatitis. HCV causes persistent infections in most cases (4) and leads to the development of chronic hepatitis and liver cirrhosis in ≈50% and 10% of cases, respectively (5). A significant proportion of patients with liver cirrhosis will also develop primary hepatocellular carcinoma (6). The prevalence of HCV infection around the world is generally between 0.4 and 2% (7–10), although a much higher level has been reported in Egypt (14%; see ref. 11). Therefore, HCV constitutes a major cause of chronic liver disease throughout the world. With the recent development of recombinant-based diagnostic assays for the detection of circulating HCV antibodies (2, 3), the risk of being infected with HCV after transfusion of blood or cellular components has been substantially reduced (12, 13). However, community-acquired infection is much more common and occurs at various frequencies in high-risk groups such as i.v. drug users, health-care workers, and sexual and household contacts of hepatitis patients, although ≈40% of cases in the United States appear to have no known risk factor for acquisition of infection (14). Thus, the development of an HCV vaccine to prevent transmission within the community is highly desirable.

HCV is distantly related genetically to both the pestiviruses and flaviviruses and, like these relatives, appears to process virion structural proteins from the N-terminal region of the polyprotein precursor encoded by the positive-stranded RNA genome (15). The host signal peptidase mediates the cleavage of a basic, presumed nucleocapsid protein (C; ≈20 kDa) from the N terminus of the polyprotein precursor followed by two glycoproteins (E1, ≈33 kDa and E2, ≈72 kDa), both of which represent potential components of the viral envelope (16–18). A variety of presumed nonstructural proteins (NS2–5) are processed from the downstream region of the polyprotein mediated, in part, by a viral protease encoded within the NS3 domain (18–21). Recently, we have expressed the entire structural gene region of HCV-1 (encompassing the complete C-, E1-, and E2-encoding genes along with part of the downstream NS2-encoding gene) in mammalian cells using a recombinant vaccinia virus (rVV) vector and have been able to copurify the E1 and E2 glycoproteins (gp33 and gp72) under nondenaturing conditions from the endoplasmic reticulum. A fraction of the purified material was shown to exist in the form of a large E1/E2 oligomeric complex (22). We now report on the efficacy of this purified preparation in vaccinating chimpanzees against experimental infection with HCV-1.

MATERIALS AND METHODS

Vaccine Preparation. A *Stu* I–*Bgl* II cDNA restriction fragment of the HCV-1 genome (nt 63 to +2901; aa 1–967; ref. 15) encoding the complete C (20 kDa), E1 (gp33 kDa), and E2 (gp72 kDa) proteins along with a C-terminally truncated NS2 product was cloned into the *Sma* I site of plasmid SC59 downstream of a hybrid early/late vaccinia promoter (S. Chakrabarti and B.M., unpublished work). BSC40 cells preinfected with wild-type WR vaccinia were transfected with the SC59 recombinant and thymidine kinase-negative recombinants, selected, and purified through three rounds of plaque purification (23). Spinner cultures of HeLa cells (10^9 cells per liter) were infected with the rVV at a multiplicity of infection of 1–10 and harvested 24 hr later by pelleting and freezing. After lysis by Dounce homogenization in hypotonic buffer, the pellet was extracted by homogenizing in 2% Triton X-100, and E1 (33 kDa) and E2 (72 kDa) were selectively copurified by successive chromatography on agarose-bound Gralanthus Nivalis-lectin (Vector Laboratories) and fast-flow S-Sepharose cation exchanger (Pharmacia). The yield of E1/E2 from 120 liters of infected HeLa cells was ≈1.5 mg (≥80% pure),

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Abbreviations: HCV, hepatitis C virus; rVV, recombinant vaccinia virus; PBL, peripheral blood lymphocytes; ALT, alanine aminotransferase; CID_{50}, dose that infects 50% of chimpanzees; RT, reverse transcriptase; C protein, nucleocapsid protein; E1 and E2, envelope glycoproteins 1 and 2; NS2–5, nonstructural proteins 2–5.
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which was used to immunize five chimpanzees (*Pan troglodytes*; L357, L653, L534, L559, and L470). Plaque assays verified the absence of live rVV in the purified preparation. Two other chimpanzees (WS176 and WS181) received an identical E1/E2 subunit vaccine derived from a 3′ truncated cDNA fragment (nt −63 to +2718; aa 1–906). There were no differences in the sizes of E1 and E2 derived from either cDNA fragment. Recombinant nucleocapsid (C) subunit was synthesized by directly expressing a cDNA fragment of HCV-1 encoding aa 2–120 in yeast. After extraction in 5 M urea and subsequent dialysis, the C protein was purified to homogeneity by successive chromatography on Q- and S-Sepharose (Pharmacia).

In two chimpanzees (L534 and L470), the above live rVV (≈5×10^8 plaque-forming units) was administered by scarification and by intradermal inoculation on week −76.

Immunization and Challenge of Chimpanzees. Chimpanzees were injected i.m. with 1–2 ml of recombinant HCV antigens combined with an oil/water micro-emulsified adjuvant according to regimens summarized in Table 1. Two chimpanzees (WS181 and WS176) received 18 immunizations over a 9-mo period, whereas the other five animals received just three injections at ≈0, 1, and 7 mo. Two of these five chimpanzees (L534 and L470) were immunized initially with the live rVV (Table 1). All chimpanzees were challenged 2–3 weeks after the final boost with ≈10 chimpanzee infectious doses (CID_{50}) of HCV-1 administered i.v. in 1 ml of autologous serum obtained before commencement of the entire vaccination schedule. The challenge virus was present in a plasma pool derived from the chronic phase of infection of chimpanzee no. 910 and shown to have a chimpanzee infectious titer of ≈10^6 CID_{50} per ml in 1985 (25). This infectivity titer was confirmed by us in 1992 immediately before challenging the vaccinated chimpanzees. (Six chimpanzees challenged i.v. with 1 ml of a 10^{-4} dilution all became infected, as did two additional chimpanzees challenged i.v. with 1 ml of a 10^{-5} dilution. Of two further chimpanzees challenged i.v. with 1 ml of a 10^{-6} dilution, only one became infected. These chimpanzees were monitored as described below.)

Monitoring of Chimpanzees. *Pan troglodytes* were maintained under standard conditions for the humane care of chimpanzees (26). Published procedures were followed in measuring serum alanine aminotransferase (ALT) levels (27), circulating HCV antibodies (2, 3), and HCV RNA (28) in extracts of plasma (drawn in EDTA), liver biopsies, and peripheral blood lymphocytes (PBL) using PCR. Liver biopsies were also examined in the electron and light microscope for evidence of ultrastructural alterations and inflammatory activity, respectively, as described (27).

RESULTS

A total of four chimpanzees were used as controls for i.v. challenge with ≈10 CID_{50} of HCV-1. HCV RNA was first detected in plasma samples obtained either 1 week (Fig. 1; L521, WS177, and L439) or 2 weeks (L663) after challenge. All of these animals were viremic at the most recently sampled times (≥32 weeks after challenge), although some had been periodically negative in PCR assays of plasma samples (Fig. 1, L521 and L663). Viral RNA was detected in extracts of liver biopsies from chimpanzee L439 obtained 8 and 12 weeks after challenge, although viral RNA was not detected in washed PBLs obtained 12 weeks after challenge, even though HCV RNA was detected in the plasma at this time (Fig. 1). All four control animals experienced acute hepatitis, as evidenced from a significant rise in serum ALT levels within 2–6 weeks after challenge. Peak ALT values were recorded 4–16 weeks after challenge (Fig. 1). Of the three animals from whom liver biopsies were examined in the electron microscope, undulating endoplasmic reticulum, membranous tubules, and rings first appeared within hepatocytes by 1–3 weeks after challenge (Fig. 1). These changes are characteristic of HCV infection of this species (27). Antibodies to the recombinant C25 antigen (a chimeric polypeptide composing parts of the C, NS3, and NS4 domains; ref. 3) appeared in all control animals (by week 11 or 12). In contrast, antibodies to the purified E1 and E2 glycoproteins remained undetectable during the follow-up period (24–36 weeks after challenge; Fig. 1). This low seroconversion rate to the E1 and E2 glycoproteins (and to the C protein) was confirmed in an expanded study of chimpanzees that were all chronically infected with either HCV-1 or the highly related H strain (ref. 29; Table 2) and is in contrast to the very high (≥95%) seroprevalence of these antibodies observed in patients from the United States with parenterally transmitted chronic non-A, non-B hepatitis (30). Even when present in experimentally infected chimpanzees, titers of antibodies to the purified E1 and E2 glycoproteins were substantially lower than those measured in infected blood donors and chronically infected patients (Table 2).

Seven chimpanzees were vaccinated with the purified E1/E2 antigens with different immunization protocols (Table 1). Reactive antibodies were induced in all of these animals but with a wide range of titers (Fig. 1). When challenged with the same dose of HCV-1 used to successfully infect all four control animals, none of the five vaccinees with the highest antibody titers at the time of challenge became viremic at any time during the follow-up period of at least 33 weeks (Fig. 1; L357, L653, L534, L559, and WS176). Plasma samples were consistently negative in PCR assays for viral RNA as were liver and PBL extracts obtained at two different times (Fig. 1; liver and PBL extracts were not tested from WS176). None of the five animals seroconverted to anti-C25 and of the four tested, none showed any hepatocyte ultrastructural changes characteristic of infection (Fig. 1). Serum ALT values remained generally within the normal range (Fig. 1), and there was little evidence for either periportal or parenchymal inflammation of the liver in contrast to the significant levels observed in three of the four control, infected animals (data not shown). Thus, these five vaccinees were effectively

Table 1. Chimpanzee immunization protocols

Chimpanzee no.	E1/E2 (gp33/gp72) subunits, μg	Adjuvant*	Vaccination schedule
WS181 and WS176	3	MF59 and MTP (10 μg)	Every other day between weeks −41 and −37 (15 times)
	30	MF59 and MTP (100 μg)	Weeks −28, −22, and −2
L653	40	MF59 and MTP (100 μg)	Weeks −34, −29, and −2
L559, L357, and L534†	40	MF75 and MTP (100–400 μg)	Weeks −31, −26, and −3
L470†‡	40	MF75 and MTP (200–400 μg)	Weeks −31, −26, and −2

All chimpanzees were challenged with ≈10 CID_{50} of HCV-1 on week 0 by i.v. inoculation.
*The compositions of MF59, MF75, and muramyl tripeptide (MTP) were as described (24).
†L534 + L470 were initially primed with live rVV on week −76.
‡Also coimmunized with 40 μg of recombinant nucleocapsid (C) subunit in same weeks.

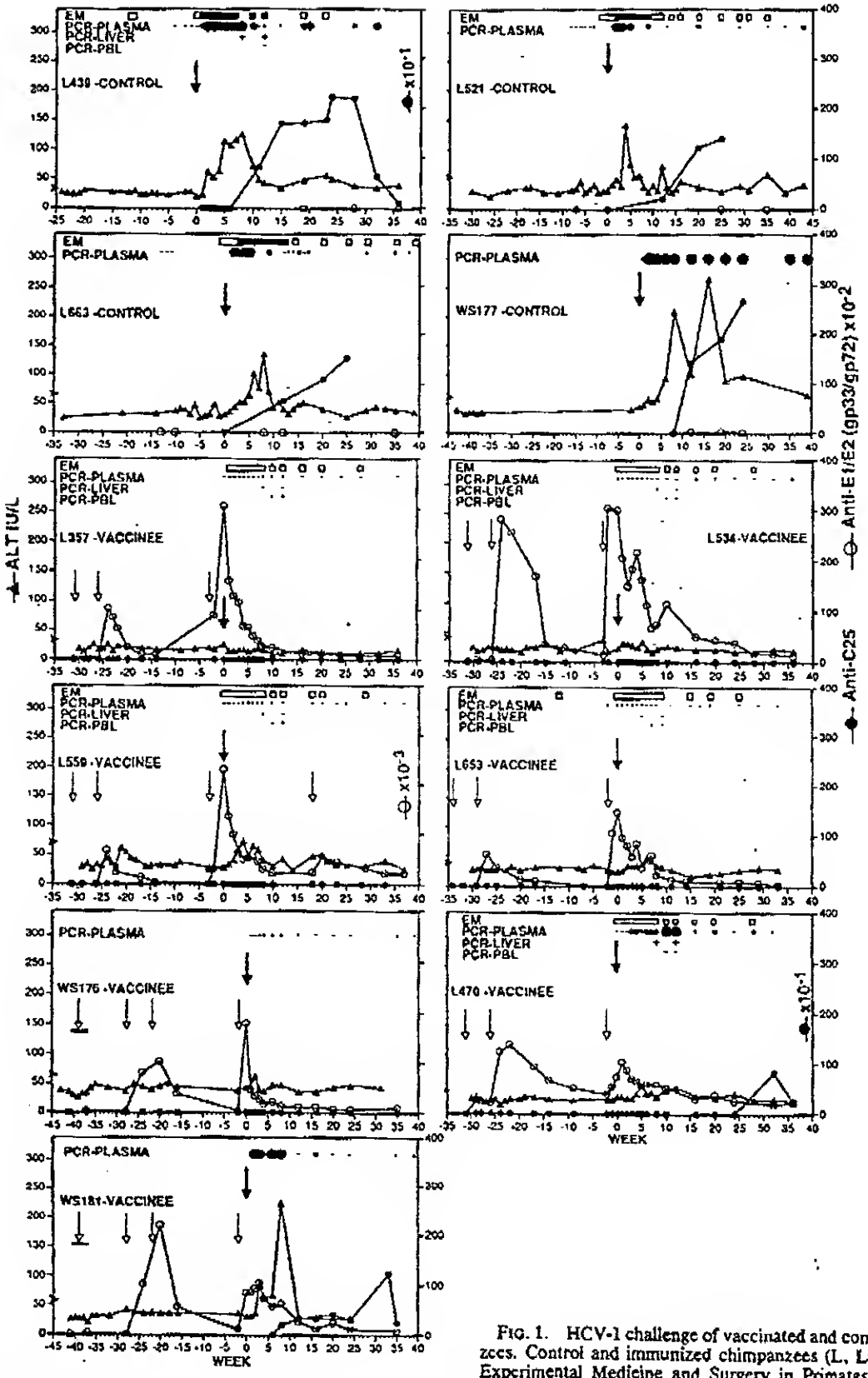

FIG. 1. HCV-1 challenge of vaccinated and control chimpanzees. Control and immunized chimpanzees (L, Laboratory for Experimental Medicine and Surgery in Primates; WS, White Sands) were challenged with ≈10 CID_{50} of HCV-1 and monitored as described. The presence (solid boxes) or absence (open boxes) of hepatocyte ultrastructural changes observed in the electron microscope (EM) is indicated. The approximate relative levels of HCV-1 RNA detected in plasma using reverse transcriptase (RT)-PCR assays are reflected by sizes of the shaded circles (open circles denote borderline positives; minus signs denote undetectable levels). The results of RT-PCR assays of liver and PBL extracts are recorded as either + or −. Note that the scales for anti-C25 and anti-E1/E2 (gp33/gp72) are different in animals L439, L470, and L559, respectively, and are expressed as the product of the ELISA OD reading in the determined linear range multiplied by the serum dilution factor. The arrows in the ALT axes indicate the mean + 3.75 SD of prechallenge values; open vertical arrows notate time of subunit vaccine administration, and the solid vertical arrow denotes viral challenge on week 0. IU/L, international units per liter.

Table 2. Prevalence of HCV antibodies in chimpanzees chronically infected with HCV-1 or HCV-H strains

	Prevalence of HCV protein domain (antigen)						
Antigen	C (C22)	E2 (gp43)	E1 and E2 (gp33/gp72)	NS3 (C33C)	NS4 (C100)	NS5 (NS5)	C, NS3, and NS4 (C25)
Ratio	4/14 (29%)	2/14 (14%)	4/10* (40%)	14/14 (100%)	12/14 (86%)	6/14 (43%)	14/14 (100%)

Antibody assays were done as described (2, 3, 30) on plasma samples from viremic chimpanzees with chronic infections of ≥36 weeks and ≤13 yr duration.

*Titers of positive samples were 23, 30, 177, and 477 (range, 0–477; mean, 71) and compared with titers of between 395 and 39,720 (mean, 4186) seen in infected blood donors and chronically infected patients (n = 25).

protected against experimental infection. In contrast, the two animals exhibiting the lowest antibody response to the vaccine at the time of virus challenge both clearly became infected and seroconverted to anti-C25 as well as to individual nonstructural proteins (L470 and WS181, Fig. 1 and data not shown). However, viremia was delayed to the third week after challenge in L470 and was generally lower than observed in the four control, infected animals (Fig. 1). Also, this animal showed no ultrastructural alterations within hepatocytes, exhibited serum ALT levels that were only minimally elevated (Fig. 1), and developed less liver inflammation than observed in three of the four control, infected animals (data not shown). Both animals have also been negative in the most recent PCR assays for viral RNA (Fig. 1).

DISCUSSION

The striking protection observed against viral challenge in five of the seven vaccinees appeared directly related to the level of the vaccine-induced antibody response. Antibody titers of ≈≥15,000 (Fig. 1) apparently resulted in protection against both infection and disease, from a challenge dose (≈10 CID_{50}) that may be representative of that transmitted in many community-acquired infections of this generally low-titer viral agent. Slight increases in serum ALT levels were observed in some of these protected animals (L559, L653, and WS176), but there were no additional markers of infection present and sensitive RT-PCR assays for viral RNA of plasma, PBL, and liver samples were all negative (Fig. 1). Conceivably, these animals might have undergone a mild, abortive infection. Alternatively, the minimal ALT elevations may have been as a result of performing frequent liver biopsies during this period, either as a direct consequence of the anesthesias and/or as a result of minimal, needle-induced liver damage.

Only one of the five protected animals was initially inoculated with live rVV (Table 1 and Fig. 1 animal L534), and the E1/E2 antigen purification scheme was shown to inactivate the original vaccinia virus used as the expression vector. In addition, two of the five protected animals were immune to vaccinia as a result of a previous inoculation with the live, wild-type WR vaccinia virus (Table 1 and Fig. 1, L357 and L653). Therefore, the observed efficacy of the subunit vaccine was clearly not dependent on priming and/or boosting of the immune response by live rVV.

Two of the seven vaccinees became infected after viral challenge; these were animals that failed to respond well to the final vaccine immunization and which, therefore, had the lowest antibody titers at the time of viral challenge (Fig. 1, L470 and WS181). However, infection was retarded and inhibited in one of these animals, as was the acute phase of hepatitis (Fig. 1, L470). Interestingly, both of these infected vaccinees were negative for viral RNA in the most recent RT-PCR assays, at which time liver functions had returned to normal (Fig. 1). Thus, these animals may have resolved their acute infections. It will be very important to confirm this hypothesis from further follow-up studies of these animals because all eight control chimpanzees experimentally infected with HCV-1 during our studies have developed chronic infections (Fig. 1 and data not shown). Thus, vaccination may stimulate the resolution of acute infection in those cases where primary infection is not prevented.

A wide range in vaccine-induced anti-E1/E2 levels was obtained among the seven animals at the time of viral challenge (Fig. 1; range, 7,300–229,920; mean, 48,391), which were generally higher than the levels observed in infected blood donors and chronically infected patients (range, 395–39,720; mean, 4186; n = 25) and which were considerably higher than the levels found in chimpanzees chronically infected with either HCV-1 or the highly related "H" strain (Table 2; range, 0–477; mean, 71; n = 10). This nonexistent or weak humoral immune response to the virion proteins observed in unimmunized, experimentally infected chimpanzees relative to infected humans (see ref. 30 and Table 2) suggests that the immunogenicity of the vaccine may, in turn, be substantially higher in humans than chimpanzees. In addition, immunogenicity data obtained by using recombinant glycoproteins of herpes simplex virus type 2 suggest that antibodies induced in human volunteers (31) are substantially more stable than in nonhuman primates (32). Thus, the stability of HCV vaccine-induced antibodies may be anticipated to be greater in humans than that observed in our vaccinated chimpanzees in which a rapid turnover was observed (Fig. 1). Future immunogenicity studies in humans will answer these important questions. It is also interesting to note that the weak or nonexistent humoral immune response to E1/E2 observed in our experimentally infected chimpanzees could account for the ready ability to re-infect chimpanzees with homologous viral challenges (33, 34).

Although the HeLa cell-derived vaccine used in the current studies was clearly efficacious, an earlier study involving the immunization of two chimpanzees with a combination of partial E1 (aa 199–330) and partial E2 (aa 404–661) antigens derived from yeast and insect cells, respectively, failed to protect both animals from infection after challenge with either ≈100 or ≈10 CID_{50} of HCV-1. However, the resulting hepatitis in one animal (10 CID_{50} challenge) was milder than that in unimmunized, infected control animals, whereas in the other (100 CID_{50} challenge), chronic infection was not established (M.H., unpublished work). Another previous study involved the immunization of one chimpanzee with live rVV followed by just a single dose of HeLa-derived E1 (gp33) and E2 (gp72). Although the induced antibody response was not high enough to prevent infection after challenge (≈100 CID_{50} of HCV-1), the resulting hepatitis was ameliorated in comparison with that in control, infected animals (M.H., unpublished work). These data indicate the importance of both antigen selection and immunization regimen in vaccine efficacy. The current study reported here did not evaluate efficacy against a challenge of >≈10 CID_{50} of HCV-1. Further investigations are required to address this potentially important issue. It should also be emphasized that the vaccinees in this study were challenged within a few weeks of the last boost at a time when antibody levels were maximal. The effects of challenging at later times on the development of both acute and chronic infection remain important issues for the future.

Another important issue relates to the observed heterogeneity of HCV. At least six related, but nonetheless distinct, genotypes have now been distinguished from phylogenetic analyses, and at least some of these virus types are composed of more than one subtype (35). The primary amino acid sequences of the putative envelope glycoprotein domains differ by up to 50% (at least), suggesting that multivalent vaccines may be required for global protection. However, the results reported here offer substantial encouragement and optimism for achieving effective control of HCV infection.

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1. Choo, Q.-L., Kuo, G., Weiner, A. J., Overby, L. R., Bradley, D. W. & Houghton, M. (1989) *Science* 244, 359–362.
2. Kuo, G., Choo, Q.-L., Alter, H. J., Gitnick, G. L., Redeker, A. G., Purcell, R. H., Miyamura, T., Dienstag, J. L., Alter, M. J., Stevens, C. E., Tegtmeier, G. E., Bonino, F., Colombo, M., Lee, W.-S., Kuo, C., Berger, K., Shuster, J. R., Overby, L. R., Bradley, D. W. & Houghton, M. (1989) *Science* 244, 362–364.
3. Chien, D. Y., Choo, Q.-L., Tabrizi, A., Kuo, C., McFarland, J., Berger, K., Lee, C., Shuster, J. R., Nguyen, T., Moyer, D. L., Tong, M., Furuta, S., Omata, M., Tegtmeier, G., Alter, H., Schiff, E., Jeffers, L., Houghton, M. & Kuo, G. (1992) *Proc. Natl. Acad. Sci. USA* 89, 10011–10015.
4. Alter, M. J., Margolis, H. S., Krawczynski, K., Judson, F. N., Mares, A., Alexander, W. J., Hu, P. Y., Miller, J. K., Gerber, M. A., Sampliner, R. E., Meeks, E. L. & Beach, M. J. (1992) *N. Engl. J. Med.* 327, 1899–1905.
5. Alter, H. J. (1989) in *Current Perspectives in Hepatology*, eds. Seeff, L. B. & Lewis, J. H. (Plenum, New York), pp. 83–97.
6. Kiyosawa, K., Tanaka, E., Sodeyama, T., Furuta, K., Usuda, S., Yousuf, M. & Furuta, S. (1990) *Jpn. J. Cancer Res.* 81, 1089–1091.
7. Zhang, H. Y., Kuramoto, I. K., Mamish, D., Sazama, K., Holland, P. V. & Zeldis, J. B. (1993) *J. Clin. Microbiol.* 31, 606–609.
8. Archer, G. T., Buring, M. L., Clark, B., Ismay, S. L., Kenrick, K. G., Purusothaman, K., Kaldor, J. M., Bolton, W. V. & Wylie, B. R. (1992) *Med. J. Aust.* 157, 225–227.
9. Sirchia, G., Bellobuono, A., Giovanetti, A. & Marconi, M. (1989) *Lancet* ii, 797.
10. Kim, Y. S., Pai, C. H., Chi, H. S., Kim, D. W., Min, Y. I. & Ahn, Y. O. (1992) *J. Korean Med. Sci.* 7, 333–336.
11. Darwish, N. M., Abbas, M. O., Abdelfattah, F. M. & Darwish, M. A. (1992) *J. Egypt. Public Health Assoc.* 67, 223–236.
12. Donahue, J. G., Muñoz, A., Ness, P. M., Brown, D. E., Yawn, D. H., McAllister, H. A., Reitz, B. A. & Nelson, K. E. (1992) *N. Engl. J. Med.* 327, 369–373.
13. Japanese Red Cross Non-A, Non-B Hepatitis Research Group (1991) *Lancet* 338, 1040–1041.
14. Alter, M. J., Hadler, S. C., Judson, F. N., Mares, A., Alexander, W. J., Hu, P. Y., Miller, J. K., Moyer, L. A., Fields, H. A., Bradley, D. W. & Margolis, H. S. (1990) *J. Am. Med. Assoc.* 264, 2231–2235.
15. Choo, Q.-L., Richman, K. H., Han, J. H., Berger, K., Lee, C., Dong, C., Gallegos, C., Coit, D., Medina-Selby, A., Barr, P. J., Weiner, A. J., Bradley, D. W., Kuo, G. & Houghton, M. (1991) *Proc. Natl. Acad. Sci. USA* 88, 2451–2455.
16. Spaete, R. R., Alexander, D., Rugroden, M. E., Choo, Q.-L., Berger, K., Crawford, K., Kuo, C., Leng, S., Lee, C., Ralston, R., Thudium, K., Tung, J. W., Kuo, G. & Houghton, M. (1992) *Virology* 188, 819–830.
17. Hijikata, M., Kato, N., Ootsuyama, Y., Nakagawa, M. & Shimotohno, K. (1991) *Proc. Natl. Acad. Sci. USA* 88, 5547–5551.
18. Selby, M. J., Choo, Q.-L., Berger, K., Kuo, G., Glazer, E., Eckart, M., Lee, C., Chien, D., Kuo, C. & Houghton, M. (1993) *J. Gen. Virol.* 74, 1103–1113.
19. Eckart, M. R., Selby, M., Masiarz, F., Lee, C., Berger, K., Crawford, K., Kuo, C., Kuo, G., Houghton, M. & Choo, Q.-L. (1993) *Biochem. Biophys. Res. Commun.* 192, 399–406.
20. Grakoui, A., Wychowski, C., Lin, C., Feinstone, S. M. & Rice, C. M. (1993) *J. Virol.* 67, 1385–1395.
21. Grakoui, A., McCourt, D. W., Wychowski, C., Feinstone, S. M. & Rice, C. M. (1993) *J. Virol.* 67, 2832–2843.
22. Ralston, R., Thudium, K., Berger, K., Kuo, C., Gervase, B., Hall, J., Selby, M., Kuo, G., Houghton, M. & Choo, Q.-L. (1993) *J. Virol.* 67, 6753–6761.
23. Karschin, A., Thorne, B. A., Thomas, G. & Lester, H. A. (1992) *Methods Enzymol.* 207, 408–423.
24. Van Nest, G. A., Stelmer, K. S., Haigwood, N. L., Burke, R. L. & Ott, G. (1992) *Vaccines '92* (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 57–62.
25. Bradley, D. W. & Maynard, J. E. (1986) *Semin. Liver Dis.* 6, 56–66.
26. Moor-Jankowski, J. & Mahoney, C. J. (1989) *J. Med. Primatol.* 18, 1–26.
27. Muchmore, E., Popper, H., Peterson, D. A., Miller, M. F. & Lieberman, H. M. (1988) *J. Med. Primatol.* 17, 235–246.
28. Gretch, D. R., Wilson, J. J., Carithers, R. L., Dela Rosa, C., Han, J. H. & Corey, L. (1993) *J. Clin. Microbiol.* 31, 289–291.
29. Ogata, N., Alter, H. J., Miller, R. H. & Purcell, R. H. (1991) *Proc. Natl. Acad. Sci. USA* 88, 3392–3396.
30. Chien, D. Y., Choo, Q.-L., Ralston, R., Spaete, R., Kuo, C., Tabrizi, A., McFarland, J., Berger, K., Tong, M., Houghton, M. & Kuo, G. (1993) *Lancet* 342, 933.
31. Straus, S. E., Savarese, B., Tigges, M., Freifeld, A. G., Krause, P. R., Margolis, D. M., Meier, J. L., Paar, D. P., Adair, S. F., Dina, D., Dekker, C. & Burke, R. L. (1993) *J. Infect. Dis.* 167, 1045–1052.
32. Burke, R. L. (1991) *Rev. Infect. Dis.* 13 (11), S906–S911.
33. Prince, A. M., Brotman, B., Huima, T., Pascual, D., Jaffery, M. & Inchauspé, G. (1992) *J. Infect. Dis.* 165, 438–443.
34. Farci, P., Alter, H. J., Govindarajan, S., Wong, D. C., Engle, R., Lesniewsi, R. R., Mushahwar, I. K., Desai, S. M., Miller, R. H., Ogata, N. & Purcell, R. H. (1992) *Science* 258, 135–140.
35. Simmonds, P., Holmes, E. C., Cha, T.-A., Chan, S.-W., McOmish, F., Irvine, B., Beall, E., Yap, P. L., Kolberg, J. & Urdea, M. S. (1993) *J. Gen. Virol.* 74, 2391–2399.

Basic immunology and its medical application

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before vaccines were available, have a higher incidence of poliomyelitis than nonpregnant women of the same age group. Many of these features modulate both natural resistance and acquired immunity.

ACQUIRED IMMUNITY

The immunity an individual develops is traditionally discussed under the heading of acquired immunity. Unlike natural resistance, which is a broad-spectrum resistance not directed against any particular pathogen, acquired immunity is expressed most typically against a specific pathogen and develops as a result of exposure to that pathogen.

Acquired immunity is the result of an adaptive reaction by two major cell groups of the host in response to a threat to the host's continued existence. Each of these cell groups is composed of two subgroups. The lymphocytes may be categorized by many characteristics as either B or T cells. The B lymphocytes, or more properly their progeny, the plasma cells, are responsible for humoral (circulatory) immunity brought about by antibodies (immunoglobulins) that they produce. The T lymphocytes serve as a major component of the cell-mediated immune system. The second cell group, the phagocytes, includes the granulocytic cells and the macrophages, derived from monocytes, as its two subgroups.

Antibody-mediated immunity

Immunity based on antibody formation is the most formidable type of immunity against most bacterial infections and bacterial toxins. Antibodies also contribute to immunity against other forms of infectious organisms. Immunoglobulin-based acquired immunity is conveniently subdivided into that which is actively acquired and that which is passively acquired.

In active immunity the individual synthesizes his own antibodies, whereas in passive immunity the individual receives antibodies either from another human or from a lower animal. Both active and passive immunity may be subdivided, depending on whether the immunity is acquired by natural or artificial means:

Acquired immunity
- A. Active
  - 1. Naturally acquired
  - 2. Artificially acquired
- B. Passive
  - 1. Naturally acquired
  - 2. Artificially acquired

Naturally acquired immunity should not be confused with natural immunity (natural resistance).

Active immunity. A degree of naturally acquired active immunity results from any infection from which a person recovers, whether the illness is serious or subclinical. During the illness the individual receives an antigenic stimulus that initiates antibody production against the specific pathogen involved. On subsequent visitation by the same or an antigenically related pathogen, the antibodies will be present to assist in the body's defenses. Because many microbes produce diseases with a high mortality, this is obviously not a very satisfactory way of developing immunity.

A major goal of immunologists interested in preventing infectious diseases has been the development of vaccines or toxoids that can be used in immunization. The immunity resulting from the injection of these immunogens is said to be artificially acquired, since it is man-made. Vaccines consist of killed or living organisms or specific fractions of them such as cell walls or capsules. Killed vaccines tend to be less effective than living vaccines. To receive the benefit of a living microorganism without the risk of its inherent pathogenicity, a great effort has been expended to develop attenuated vaccines. Attenuated vaccines consist of viable but

weakened organisms, which produce only a mild infection in normal persons. Those with an immunodeficiency disease should not receive attenuated vaccines, since organisms in the vaccine will continue to multiply and cause a serous disease in such persons. Table 2-2 lists several killed and attenuated vaccines used in human medicine.

Toxoids, which are also excellent antigens, consist of the detoxified but still antigenically active poisons excreted by certain bacteria. The change from *toxin* to *toxoid* can be made simply, often by merely adding formaldehyde to the toxin. Antibodies against toxoids are fully reactive with the native toxin. Toxoids are considered to be one of the most successful immunizing reagents and produce a strong immunity against diseases caused by toxigenic bacteria (e.g., tetanus and diphtheria). Antibodies against toxoids are known as antitoxins (Table 2-2).

Passive immunity. Passive immunity may also be acquired by natural or artificial means. Naturally acquired passive immunity usually refers, at least in humans, to the transplacental passage of antibodies from the mother to her unborn child during the latter part of pregnancy. This is caused almost entirely by IgG, since IgM, IgD, and IgA do not cross the placental barrier. Antibodies may also be passed from a woman to her newborn child through colostrum, which contains secretory IgA and secretory IgM but very little of the other immunoglobulins. Since the digestive system of the newborn infant is poorly developed, breast-fed babies can absorb these immunoglobulins directly from the gastrointestinal system. Even if not absorbed, these antibodies may passively coat the infant's digestive tract and ward off intestinal infections. Naturally this system cannot operate in bottle-fed babies, and it is probably not too effective in breast-fed babies because the mother gradually reduces her secretion of these antibodies and the baby's digestive system soon begins to catabolize them.

Passive immunity of the artificial type re-

Table 2-2. Immunizing reagents for leading bacterial and viral infections

Disease	Etiologic agent	Nature of immunizing agent
Bacterial		
Cholera	*Vibrio cholerae*	Killed bacteria
Diphtheria	*Corynebacterium diphtheriae*	Toxoid
Meningitis	*Neisseria meningitidis*	Capsular polysaccharide
Pertussis (whooping cough)	*Bordetella pertussis*	Killed organisms
Plague	*Yersinia pestis*	Killed bacteria
Pneumonia	*Streptococcus pneumoniae*	Capsular polysaccharide
Tetanus	*Clostridium tetani*	Toxoid
Tuberculosis	*Mycobacterium tuberculosis*	Attenuated BCG (bacille Calmette-Guérin)
Tularemia	*Francisella tularensis*	Live attenuated or killed organism
Typhoid fever	*Salmonella* species	Killed bacteria of three major species
Viral		
Influenza		Inactive
Measles		Attenuated
Mumps		Attenuated
Poliomyelitis		Inactive or attenuated
Rabies		Inactive
Rubella		Attenuated
Smallpox		Attenuated
Yellow fever		Attenuated

fers to the original production of antibodies in some other individual (either human or lower mammal) and the acquisition of these antibodies through a needle and syringe. Injections of hyperimmune serum, antiserum, antitoxin, γ-globulin, or immune serum are examples of this type of immunization. Several pharmaceutical companies are involved in the large-scale production of antibodies in horses, cows, and other animals by active immunization so that these antibodies may be used later to prevent human diseases by passive immunization. A more limited program for the hyperimmunization of humans is designed for the same purpose.

A special form of antibody-related immunity is exemplified by adoptive immunity wherein antibody-forming cells are transferred from one person to another. This is practiced only when the recipient has a genetic immunodeficiency disease.

Comparison of active and passive immunity. Active and passive immunity must be compared on a broader basis than whether a person makes his own antibodies; it is also critical to know whether immunity has been naturally or artificially acquired. There are other important and sometimes subtle differences between the two, most of which are summarized in Table 2-3.

The comparative effectiveness of active and passive immunity is heavily weighted in favor of the former. This is related to several factors, one of which is the duration of the immunity. Active immunity is known to persist for relatively long periods, usually years, without reactivation through booster immunization. This is true because once lymphocytes and plasma cells are activated to produce antibodies, they continue to do so for the lifetime of the cell. In passive immunization, this does not happen. The injected antibodies are removed from the circulation without internal replacement, and the immunity can then be directly correlated with the amount of antibodies injected and their half-life. The half-life of human antibodies in a human is about 30 days, but the half-life of equine or bovine antibodies in a human is about 7 days. It must be remembered that foreign serum proteins, including γ-globulins, function much like foreign microorganisms in the recipient, who then makes antibodies against the antibodies. This results in their speedy elimination.

Of course, passive immunity can be quickly restored or maintained by repeated injections of the antiserum. This is satisfactory when human antiserum is employed, but not when antiserum from a foreign species is used. In this instance the possibility of anaphylaxis or serum sickness is great (Chapter

Table 2-3. Comparison of active and passive immunity brought about by immunoglobulins

	Active immunity	Passive immunity
Source	Self	Another human or a lower animal
Effectiveness	High	Moderate to low
Method	Disease itself, clinical or subclinical	Maternal transplacental transfer
	Immunization by (a) killed or attenuated vaccines or (b) toxoids	Injection
Time to develop	5 to 14 days	Immediate on injection
Duration	Relatively long, perhaps years	Relatively short, a few days to several weeks
Ease of reactivation	Easy (by booster)	Dangerous, possible anaphylaxis
Use	Prophylactic	Prophylactic and therapeutic

10), anaphylaxis itself being a life-threatening proposition. On the other hand, the reactivation of active immunity by "booster" injections of the vaccine or toxoid is a comparatively risk-free method and one that is commonly utilized.

It must be pointed out that high-efficiency active immunity requires 5 to 14 days after the primary immunization to develop. This is the time it takes for protective quantities of antibodies to appear in the serum. After booster injections of antigen, only 1 to 3 days are required. With passive immunization, protection is provided immediately on completion of the injection.

Active immunization is usually restricted to prophylactic (preventive) applications by which the person receives the immunization far in advance of exposure to the infectious agent. Under special conditions (e.g., rabies, smallpox) when the incubation time of the disease is longer than the time required for antibody formation, it is possible for the individual to be immunized after exposure. Passive immunization can also be used prophylactically, that is, prior to or immediately after exposure, in individuals who have not been actively immunized. Passive immunization (but not active immunization) can also be used therapeutically. Unfortunately this is a relatively inefficient process because the organisms or their toxins may have created considerable undetected cell damage prior to the administration of the antiserum. None of this damage is reparable by antiserum alone.

Primary and secondary immune response. The initial burst of immunoglobulin formation after the first exposure to a vaccine, or to any antigen of which it is composed, is referred to as the primary immune response (Fig. 2-2). This term is used even if protection does not result from the antigen exposure (e.g., the exposure to ragweed pollen or to allergens). Not until 5 to 14 days after this primary antigen exposure is it possible to detect immunoglobulins in the blood. This does not mean that this amount of time passes before antibody production begins; it means only that it takes that long before the quantity of antibody in the blood is sufficient to be detected easily. The amount of antibody in the blood increases gradually over

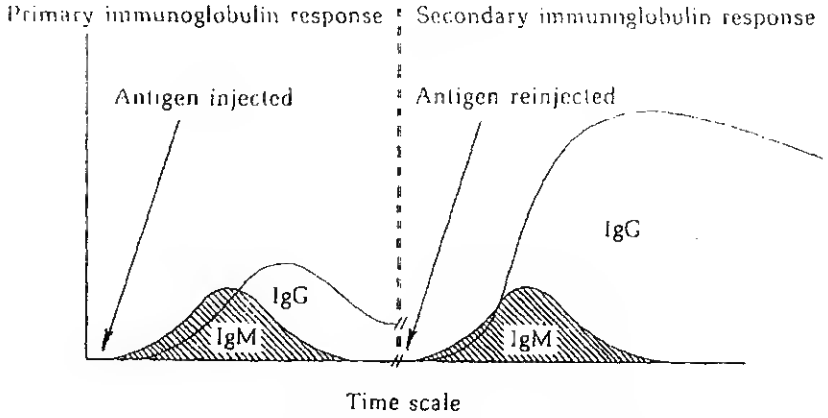

Fig. 2-2. Difference in IgG response to primary and secondary exposure to antigen indicates why it is termed the memory antibody. IgM, illustrated to exhibit no "memory," usually shows a slight anamnestic response.